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ON THE USE OF A MONILIA VACCINE IN THE TREATMENT OF SPRUE

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Sprue has been studied for years and numerous theories as to its etiology have been advanced, but without sufficient proofs to cause their acceptance by workers in tropical medicine.

Ashford has devoted much time to the study of this disease, with the result of the discovery of a monilia, which could be isolated from the tongue and feces of sprue patients and also from the tissues of persons dying of the disease. For this monilia he adopted the term *Monilia psilosis*. The organism and its etiologic relationship to the disease, including the production of sprue symptoms in animals, have been previously described. My studies have been serologic and I have found, using *Monilia psilosis* antigens, complement fixation in cases of sprue. These cases had been diagnosed and the presence of the monilia determined culturally by Ashford. During the experiments on the toxins of the *Monilia psilosis* evidence indicating specificity was furnished by the production of immunity in animals. This immunity was produced by the injection of killed cultures, and it was hoped from this that a vaccine might be made having a curative value.

DIAGNOSIS AND TREATMENT

In sprue the diagnosis is based on the following findings — sore tongue, excessive intestinal fermentation, light foamy diarrhea, diminution in size of liver, emaciation.

In cases in which a positive diagnosis of sprue could be made on these points, Ashford isolated *Monilia psilosis* from the tongue and feces of the patients, and the complement fixation test was positive in the samples of blood examined.

In sprue, numerous remedies have been tried and abandoned, but diet has remained the most reliable and successful means of treatment. Various diets are used, the effort being to secure one composed of food which would be easily assimilated and which would tend to inhibit gastro-intestinal symptoms. The milk diet, when it could be properly

carried out, gave the most favorable results, while the fruit and milk diet, the meat diet, and mixed diets have been favorably reported in some instances. Results from dietary treatment, however, are obtained only very slowly and sometimes require a period of months or even years. It has been the universal experience that it is very difficult to keep a patient on one diet for an extended period. Then, too, in certain places, particularly in tropical countries, it may be almost impossible for a patient to procure the prescribed diet.

For these reasons, constant endeavor to find a more satisfactory treatment and one requiring a shorter period of time, and especially a specific remedy, has gone on unceasingly.

Experiments demonstrated that endotoxins were probably the active toxic substances elaborated by *Monilia psilosis*. An emulsion of the monilia was sterilized and injected into guinea-pigs. These animals developed a relative immunity to injections of living cultures. There seemed therefore to be a possibility that a specific vaccine might be developed.

PREPARATION AND INJECTION OF THE VACCINE

Cultures of *Monilia psilosis* which had been isolated by Ashford from a fatal case of sprue were used. Inoculations were made on Sabouraud's medium and incubated 4 days at 37 C. An emulsion was then made of the 4 day cultures, using 1 culture in 10 c.c. of distilled water, and shaken in a machine for 2 hours, after which it was heated to 37 C. for 5 days, which allowed autolysis to take place. After the addition of phenol (to make $\frac{1}{2}\%$ of the volume) the vaccine was sterilized at 56 C. for 1 hour. Cultures were now made to test it for sterility, and animals were injected with from 5 to 8 c.c. If the animals showed no signs of infection in 2 weeks and the cultures showed no growth, the vaccine was used. The difficulty in preparing the vaccine was due to the high thermal death point of the monilia, which is 70 C. Autolysis was used to lower the thermal death point.

Owing to the difficulty of counting the number of monilia in the emulsion, and because of the polymorphic forms of the organism, the following method of standardization was adopted:

Ten c.c. of the emulsion was put in a Purdy graduated centrifuge tube and centrifuged at a medium speed for 5 minutes, after which a reading was taken. The amount of sediment composed of monilia should be 1% in the standard of emulsion used. The primary dose of this emulsion given was about 0.05 c.c.

There was no guide to the dosage of the vaccine thus prepared except its injection into normal persons and patients having sprue. For this purpose a primary dose of 0.05 c.c. of a 1% solution of the emulsion was used.

In the series, the dosage of the vaccine to be used was determined by its effect on normal persons and patients having sprue. Ten normal persons were injected with doses from 0.1 c.c. to 0.2 c.c. and the reactions observed; 0.2 c.c. produced a fairly severe local reaction which lasted about 4 days and subsided, without gastro-intestinal symptoms being produced. In normal persons or those suffering with other diseases doses of vaccine up to 0.6 c.c. have been administered without producing any other than a local reaction.

Five patients having sprue were injected with 0.05 c.c. to 0.1 c.c. and similarly observed. The injection of 0.05 c.c. produced a local moderate reaction and an increase of gastro-intestinal symptoms. The injection of 0.1 c.c. produced a severe reaction, the site of injection becoming deep red and inflamed for an area of 1 cm. Around this an area of erythema extended for 7.5 cm. The tongue became very sore, and the diarrhea increased with a great deal of flatulence, accompanied by a rise in temperature from 2-4 F. These symptoms were severe enough to require the patients to rest. They complained of depression and weakness.

The most marked features of the reactions noted were the increased soreness of the tongue and aggravation of gastro-intestinal symptoms.

The reaction was most severe on the 2nd day after the injection and commenced to subside about the 3rd to the 5th day. Considering the degree of local and general reactions that were produced, especially in very severe cases of sprue in which there is marked disability, it seems preferable to administer a primary dose of not more than 0.5 c.c.

The second injection was given 8 to 10 days after the first injection. The time of this administration should, it is believed, vary with the condition of the patient. The dosage given was about twice the first dose, from 0.15 c.c. to 0.2 c.c. being injected. The interval of the dosage depended on the condition of the patient, the 10 day interval being the most preferable.

In most cases the second reaction was not as severe as the first. A moderate local reaction was, however, produced which lasted 4-5 days, accompanied by an increased soreness of the tongue, slight flatulence, fever of 1-3 F. and slight diarrhea. These symptoms subsided rapidly, and after 5 days improvement was noted in the general condition of

the patients. There was a marked increase in appetite and the patients usually gained several pounds in weight. The intestinal symptoms were notably diminished.

The third injection was given 10 days after the second, from 0.5 to 0.6 c.c. being given. The reaction which followed was very mild, subsiding after about 2 days' duration. The intestinal symptoms were very slight.

The fourth injection was given about 12 days after the third and practically no reaction resulted, except a slight local induration and redness. The amount injected was 5.7 c.c. The general health of the patients by this time was greatly improved and in most cases they were gaining in weight, about 2 pounds per week. The symptoms of sprue had greatly diminished.

The fifth injection of 0.9 c.c. was given 14 days later and there was usually a slight or no gastro-intestinal reaction. The patient complained of weakness which lasted for 1 day. The diet was increased and the patients were allowed to have sugar and cereals in small amounts.

The sixth injection of 1.4 c.c. given 14 days after the 5th produced nothing but a localized reaction which lasted 2-3 days. The sprue symptoms were absent and the patient was allowed all kinds of food, including sugars and all carbohydrates. The patients were kept under observation for 2 months and no sprue symptoms noted.

HISTORY OF A TYPICAL CASE

A woman, of middle age, has had sprue for more than 2 years. Has all the typical symptoms, including severe anemia, sore tongue and intestinal disturbances. Complement fixation test for sprue, 100% positive. *Monilia psilosis* isolated from tongue and feces. She has been under diet treatment both in Porto Rico and the United States without material improvement.

The 1st injection of vaccine, 0.1 c.c., was followed by a very severe reaction, which kept her in bed. The local reaction following consisted in a reddened area with swelling and soreness of the arm. The constitutional symptoms were well marked and simulated an attack of acute sprue. The tongue had the increased soreness and reddening. There was an increase of flatulence and gastro-intestinal symptoms with a slight rise in temperature of 2 F. The symptoms abated after 5 days, leaving the patient with a feeling of weakness.

The 2nd injection of 0.2 c.c. was given 10 days after the 1st injection. Reaction, similar but less severe, followed, the duration being 4 days. At the termination of reaction, great improvement was noted by the patient. Gastro-intestinal and tongue symptoms were especially improved. Patient gained 9 pounds in weight in 3 weeks. The 3rd injection of 0.4 c.c. was given 18 days after the 2nd. Very slight reaction of 2 days' duration. Great improvement in general condition of patient; gained 3 pounds.

The 4th injection of 0.8 c.c. was given 10 days after the 3rd. A slight reaction followed and there was a slight flatulence which lasted 1 day. The diet of the patient was increased and there was a steady gain in weight.

The 5th injection was given 14 days later. Only a slight local reaction resulted. The patient weighs more than she has in the last 2 years and for the past 3 months since the last injection has been apparently well and has been on full diet including carbohydrates and sugars without any symptoms. *Monilia psilosis* cannot be found in scrapings from the tongue or feces. Complement fixation test is slightly positive.

RESULTS OF TREATMENT

Of 81 cases, 62 patients have completed the treatment. All cases were diagnosed by Ashford and *Monilia psilosis* was isolated from the feces. In all these cases the complement fixation test was positive. The diet was regulated so as to fit each individual case. The only medication given was to stimulate digestion. Sprue being confused with other diseases and being often complicated, a careful positive diagnosis was made in each case. Cases of sprue without complications responded most readily to the treatment. Americans responded very quickly to the treatment and required a shorter time than Porto Ricans.

Of 62 patients treated, 49 patients were discharged cured, 12 were improved, and 1 died.

SUMMARY OF CASES TREATED

Cases Cured		Cases Improved		Cases Unimproved	
Cases	Number of Injections	Cases	Number of Injections	Cases	Number of Injections
10	8	1	7	1	5
11	7	2	6	Died of acute nephritis	
11	6	1	5		
10	5	7	4		
		1	3		
49 Cured	12 Improved			1 Death	

The results of the vaccine treatment are very encouraging, and all patients under treatment have shown great improvement. In noting the results of the treatment, the mental attitude of the patients has been considered to ascertain whether the injections and reactions produced a psychic improvement only. In certain cases there was a distinct abatement of sprue symptoms after the 3rd injection and instead of a diarrhea, constipation resulted. The most notable feature was the gain in weight, which was progressive from week to week. There was, in all cases, a great improvement in the general physical condition of the patient, and the disappearance of the monilia from the feces. The results of this treatment are very favorable, and it is believed that *Monilia psilosis* vaccine should be tried in other localities where sprue is present.

CASE HISTORIES

1.—Mrs. D., American, aged 48 years, weight 82 pounds; sick 2 years; diagnosis, sprue; all symptoms present; on a diet for one year; monilia isolated from tongue and feces; complement fixation test positive.

February 5, monilia vaccine, 0.1 c.c.; severe reaction; February 15, 0.2 c.c.; moderate reaction. March 1, 0.4 c.c.; moderate reaction; March 17, 0.6 c.c.; moderate reaction. April 3, 0.8 c.c.; no reaction; April 25, 1.2 c.c.; slight reaction; after 3rd injection patient was placed on a full diet, gained weight rapidly; all sprue symptoms had disappeared after 4th injection.

June 11, patient has no symptoms, weighs 110 pounds; monilia absent from tongue and feces.

July 1, complement fixation test negative; discharged recovered.

2.—Mrs. S., American, weighs 96 pounds; sick for nearly 1 year; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test positive.

March 23, monilia vaccine, 0.05 c.c.; moderate reaction; March 29, 0.2 c.c.; severe reaction. April 7, 0.4 c.c.; moderate reaction; April 21, 0.6 c.c.; slight reaction. May 4, 0.8 c.c.; no reaction; after 3rd injection patient was put on full diet; slight symptoms remained.

June 14, patient shows no symptoms; weighs 115½ pounds; discharged cured; feces negative for monilia.

3.—B. G., woman, Porto Rican, aged 39, weight 79 pounds; sick for over 8 months with sprue; has had previous attacks; complement fixation test positive.

March 23, monilia vaccine, 0.1 c.c.; severe reaction; March 29, 0.2 c.c.; moderate reaction. April 3, 0.4 c.c.; severe reaction. May 1, 0.8 c.c.; mild reaction; May 21, 1 c.c.; slight reaction.

June 11, all symptoms have disappeared; patient on full diet without any gastric disturbances; weight 97 pounds; vaccine was administered at too short intervals and great depression followed the reactions; discharged cured.

4.—G. A., woman, Porto Rican, weight 142 pounds; sick for over 2 years; symptoms not very severe; has not lost much weight; diagnosis, sprue; monilia isolated from tongue and feces. Complement fixation test positive.

March 27, monilia vaccine, 0.1 c.c.; moderate reaction. April 7, 0.2 c.c.; moderate reaction; April 17, 0.4 c.c.; moderate reaction; April 28, 0.6 c.c.; slight reaction. May 26, 0.8 c.c.; no reaction. June 5, 1.2 c.c.; no reaction. No sprue symptoms present; patient has been on full diet; weighs 160 pounds; discharged cured.

5.—M. P., woman, Porto Rican, aged 35, weight 78 pounds; has had sprue for several years; monilia isolated from tongue and feces; complement fixation test positive.

March 21, monilia vaccine, 0.1 c.c.; moderate reaction; March 21, 0.3 c.c.; very severe reaction. April 14, 0.6 c.c.; moderate reaction. May 1, 0.8 c.c.; slight reaction; May 21, 1 c.c.; no reaction. June 4, 1.2 c.c.; no reaction.

July 7, all sprue symptoms absent; patient on full diet without gastric disturbance; weight 96 pounds; discharged cured.

6.—Ske. E., woman, Porto Rican, aged 64, weight 67 pounds; sick for 2 years; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test positive.

April 15, monilia vaccine, 0.1 c.c.; very severe reaction. May 1, 0.2 c.c.; moderate reaction; May 15, 0.3 c.c.; moderate reaction; May 31, 0.5 c.c.; slight reaction. June 14, 0.7 c.c.; no reaction; June 29, 0.9 c.c.; no reaction.

July 14, no symptoms present; on full diet; appetite good; digestion good; weighs 79 pounds; discharged cured.

7.—A. A., man, Porto Rican, aged 19 years, weight 80 pounds; sick for 1½ years; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test positive.

May 1, monilia vaccine, 0.1 c.c.; severe reaction, fever, vomiting and diarrhea; May 11, 0.2 c.c.; moderate reaction; May 19, 0.35 c.c.; moderate reaction; May 28, 0.6 c.c.; moderate reaction. June 9, 1.2 c.c.; slight reaction; June 22, 1.4 c.c.; no reaction.

July 3, no symptoms of sprue present; on full diet; no gastric disturbance; digestion good; weighs 106 pounds; discharged recovered.

8.—V. J. A., man, Porto Rican, aged 31 years, weight 92 pounds; sick for 9 months; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test positive.

May 11, autogenous monilia vaccine, 0.05 c.c.; reaction; May 18, 0.1 c.c.; reaction; May 25, 0.2 c.c.; reaction. June 1, 0.4 c.c.; slight reaction; June 11, 0.6 c.c.; no reaction; June 25, 0.8 c.c.; no reaction; all sprue symptoms have disappeared; patient on full diet; weighs 105 pounds; discharged recovered; no gastric symptoms present.

9.—R. J., boy, Porto Rican, aged 16 years, weight 68 pounds; sick for about 3 months; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test is positive.

April 30, monilia vaccine, 0.05 c.c.; severe reaction. May 7, 0.1 c.c.; moderate reaction; May 14, 0.2 c.c.; severe reaction; May 21, 0.4 c.c.; moderate reaction. June 2, 0.6 c.c.; moderate reaction; June 13, 0.8 c.c.; no reaction.

July 18, sprue symptoms absent; no gastric symptoms; patient on full diet; weighs 79 pounds; discharged recovered.

10.—M. M., woman, Porto Rican, aged 27, weight 101 pounds; sick for 7 years with sprue symptoms; treated by a great many physicians; has been on dietetic treatment which caused a temporary improvement; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test positive.

March 21, monilia vaccine, 0.1 c.c.; reaction. April 2, 0.2 c.c.; severe reaction; April 17, 0.4 c.c.; reaction. May 1, 0.6 c.c.; slight reaction; May 15, 0.8 c.c.; no reaction; May 23, 1 c.c.; no reaction; weighs 118 pounds.

July 19, patient has no sprue symptoms; has full diet; weighs 121 pounds, which is more than she has weighed in the last 6 years; discharged recovered.

11.—P. de P., Spaniard, aged 34 years, weight 86 pounds; sick for 3 years with sprue symptoms; has been on dietary treatment which caused some improvement; monilia isolated from tongue and feces.

May 4, monilia vaccine, 0.05 c.c.; reaction; May 11, 0.1 c.c.; moderate reaction; May 18, 0.2 c.c.; moderate reaction. June 2, 0.6 c.c.; moderate reaction; June 11, 0.8 c.c.; moderate reaction. July 3, 1.1 c.c.; slight reaction; July 18, 1.3 c.c.; no reaction; patient has no sprue symptoms; has full diet; no gastro-intestinal symptoms present; weighs 94 pounds; discharged cured.

12.—A. P., Porto Rican, man, aged 55 years, weight 172 pounds; acute attacks of sprue for last 5 years; dietary treatment gave temporary relief; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test positive.

April 23, monilia vaccine, 0.1 c.c.; reaction. May 1, 0.2 c.c.; reaction; May 7, 0.3 c.c.; slight reaction; May 23, 0.6 c.c.; moderate reaction. June 5, 1.2 c.c.; moderate reaction; June 26, 1.5 c.c.; slight reaction. July 10, 1.5 c.c.; no reaction; July 26, 1.7 c.c.; no reaction; no symptoms of any kind; has very

good appetite; on full diet; weighs 184 pounds; monilia absent in feces and scrapings of tongue; discharged recovered.

13.—A. A., woman, Porto Rican, aged 56 years, weight 130 pounds; sick for last 3 years with acute attacks of sprue; was sent to the United States and returned without relief; monilia isolated from feces.

May 9, monilia vaccine, 0.05 c.c.; mild reaction; May 17, 0.1 c.c.; severe reaction; May 23, 0.2 c.c.; moderate reaction. June 1, 0.3 c.c.; moderate reaction; June 12, 0.6 c.c.; moderate reaction; June 25, 0.8 c.c.; no reaction. July 9, 1 c.c.; no reaction.

July 15, no sprue symptoms present; patient weighs 137 pounds; monilia absent from feces; discharged cured.

14.—R. M., Porto Rican, aged 48 years, weight 108 pounds; sick for 6 years with stomach trouble; has been on dietary treatment; monilia isolated from tongue and feces.

May 1, monilia vaccine, 0.05 c.c.; slight reaction; May 8, 0.1 c.c.; slight reaction; May 16, 0.2 c.c.; severe reaction; May 23, 0.3 c.c.; severe reaction. June 1, 0.4 c.c.; mild reaction; June 11, 0.6 c.c.; slight reaction; June 25, 0.8 c.c.; no reaction. July 17, 1 c.c.; no reaction; patient on full diet; no sprue symptoms present; weighs 117 pounds; discharged recovered.

15.—V. A., man, Porto Rican, aged 45 years, weight 100 pounds; sick 1 year with typical sprue symptoms; monilia isolated from tongue and feces.

May 17, monilia vaccine, 0.05 c.c.; severe reaction; May 26, 0.15 c.c.; severe reaction. June 4, 0.2 c.c.; mild reaction; June 13, 0.5 c.c.; moderate reaction. July 1, 0.8 c.c.; moderate reaction; patient on full diet; no sprue symptoms; weighs 118 pounds; discharged recovered; monilia absent from feces.

16.—*Sprue with Pellagra*. R. C., woman, Porto Rican, aged 39 years, weighs 78 pounds; sick with severe gastric disturbance, burning of the stomach, diarrhea; gives history of having an eruption on ears and neck; hands show evidence of pellagrous eruption; could not eat meat because it would create gastric disturbance; sprue symptoms also present; monilia isolated from tongue and feces; complement fixation test positive; diagnosis, sprue and pellagra.

April 9, monilia vaccine, 0.05 c.c.; severe reaction; April 19, 0.1 c.c.; severe reaction. May 1, 0.2 c.c.; severe reaction. Ulceration developed at site of 2nd injection; patient has not gained in weight, is very weak; patient is put on a heavy protein diet and medication given to aid digestion.

May 22, monilia vaccine, 0.4 c.c.; moderate reaction; May 29, 0.6 c.c.; moderate reaction. June 10, 0.8 c.c.; mild reaction. July 6, 1 c.c.; mild reaction; patient weighs 90½ pounds; no sprue symptoms present; general condition greatly improved; mentality improved; discharged recovered.

17.—*Sprue with Pellagra*. M. P., a woman, Porto Rican, aged 35 years, weight 78 pounds; has been sick for last 4 years; an eruption has occurred on hands and feet every summer for last 3 years; complains of constant burning in throat and stomach; tongue is very smooth and red; sprue symptoms also present; monilia isolated from feces; diagnosis, sprue with pellagra; special diet of high protein content prescribed and requested to eat beans at least 2 times a day.

April 8, monilia vaccine, 0.05 c.c.; slight reaction; April 19, 0.1 c.c.; severe reaction. May 1, 0.2 c.c.; severe reaction; May 11, 0.4 c.c.; moderate reaction; May 21, 0.9 c.c.; moderate reaction. June 4, 1.2 c.c.; slight reaction.

July 7, patient has slight burning in stomach; all other symptoms absent; weighs 96 pounds; discharged recovered.

18.—*Sprue with Glycosuria*. H. M. E. B., man, American, aged 45 years; sick for past 2 years with slight attacks of sprue; complains of constant headache and dizziness; has lost very little weight; examination of urine shows 1.8% sugar present; monilia isolated from tongue and feces; complement fixation test is positive; patient was put on a diet from which carbohydrates were eliminated.

April 21, monilia vaccine, 0.05 c.c.; mild reaction; sugar in urine, 1.6%. May 9, 0.1 c.c.; severe reaction, sugar in urine, 1.8%; May 21, 0.2 c.c.; moderate reaction, sugar in urine, 1.6%; May 31, 0.6 c.c.; moderate reaction, sugar in urine, 0.936%. June 15, 1 c.c.; slight reaction, sugar in urine, 0.85%; June 28, 1.2 c.c.; very slight reaction.

July 11, patient greatly improved; no sprue symptoms present; urine is free from sugar.

July 21, improvement continues; sugar absent from urine; diet has been increased by addition of carbohydrates, including small amounts of sugar; discharged recovered.

19.—*Sprue with Tuberculosis*. O. F., man, Porto Rican, aged 21 years, weight 120 pounds; sick for over 2 years with cough and night sweats; has sprue symptoms; examination of sputum shows tubercle bacilli and feces show presence of monilia.

May 12, monilia vaccine, 0.05 c.c.; severe reaction; May 19, 0.1 c.c.; severe reaction. June 1, 0.15 c.c.; moderate reaction; June 25, 0.3 c.c.; moderate reaction. July 6, 0.4 c.c.; moderate reaction; July 17, 0.6 c.c.; slight reaction; July 28, 0.8 c.c.; no reaction.

July 30, sprue symptoms absent; has full diet; general physical condition improved; weighs 128½ pounds; tubercle bacilli still present in sputum.

20.—*Sprue with Ankylostomiasis*. J. S., man, Porto Rican, aged 31 years; weight 110 pounds; sick for 2 years with stomach trouble; during the last 3 months developed sore tongue and severe diarrhea; monilia isolated from feces; also eggs of uncinaria; all clinical symptoms of sprue present.

May 8, monilia vaccine, 0.05 c.c.; reaction; May 15, 0.1 c.c.; moderate reaction; May 22, 0.2 c.c.; moderate reaction; May 29, 0.3 c.c.; moderate reaction. June 8, 0.5 c.c.; moderate reaction; up to this time patient has not gained in weight; sprue symptoms improved; thymol given for uncinariasis.

June 20, monilia vaccine, 0.7 c.c.; slight reaction. July 3, 0.8 c.c.; no reaction; case has greatly improved; gain in weight noticeable; weighs 116 pounds; feces negative for ankylostoma; patient has full diet; no sprue symptoms present; discharged recovered.